

# Quality control by a mobile molecular workshop: quality versus quantity

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Ribosome is a molecular machine that moves on a mRNA track while, simultaneously, polymerizing a protein using the mRNA also as the corresponding template. We define, and analytically calculate, two different measures of the efficiency of this machine. However, we argue that its performance is evaluated better in terms of the translational fidelity and the speed with which it polymerizes a protein. We define both these quantities and calculate these analytically. Fidelity is a measure of the quality of the products while the total quantity of products synthesized in a given interval depends on the speed of polymerization. We show that for synthesizing a large quantity of proteins, it is not necessary to sacrifice the quality. We also explore the effects of the quality control mechanism on the strength of mechano-chemical coupling. We suggest experiments for testing some of the ideas presented here.

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## I. INTRODUCTION

For cyclic machines with *finite cycle time*, the *efficiency at maximum power output* [1–3] is a suitable indicator of its performance [4]. For molecular motors, the *Stokes efficiency* [5, 6] is an alternative measure of performance. However, not all machines are designed for the sole purpose of performing mechanical work. In this paper we consider a class of machines whose main function is to synthesize a hetero-polymer, subunit by subunit, using another hetero-polymer as the corresponding template.

The conceptual framework that we develop here is generally applicable to all machines which drive template-dictated polymerization. But, for the sake of concreteness, we formulate the theory here for a specific machine, namely, the ribosome [7]; it polymerizes a protein using a messenger RNA (mRNA) as the corresponding template and the process is referred to as *translation* (of genetic message). The subunits of a mRNA are nucleotides, whereas those of a protein are amino acids. The correct sequence of amino acids to be selected by the ribosome is dictated by the corresponding sequence of the codons (triplets of nucleotides). The ribosome also uses the mRNA template as the track for its movement. In each step, the ribosome moves forward by one codon on its track, and the protein gets elongated by one amino acid. Ribosome has a quality control system to minimize translational error by rejecting incorrect incoming amino acid subunits.

For a ribosome, the average speed  $V$  of polymerization of a protein and the fidelity  $\phi$  of translation, rather than efficiency and power output, are the primary indicators of its performance. We define these quantities, as well as a few others, quantitatively and calculate these analytically. Contrary to naive expectations, we show

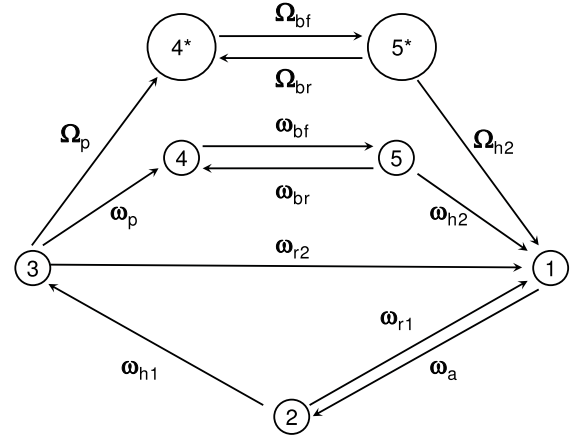


FIG. 1: Pictorial depiction of the full chemo-mechanical cycle of a single ribosome (see the text for details).

that a higher rate of elongation of the protein does not necessarily sacrifice the translational fidelity.

## II. THE MODEL

A ribosome consists of two interconnected parts called the large and the small subunits. The small subunit binds with the mRNA track and decodes the genetic message of the codon, whereas the polymerization of the protein takes place in the large subunit. The operations of the two subunits are coordinated by a class of adapter molecules, called transfer RNA (tRNA). One end of a tRNA helps in the decoding process by matching its anti-codon with the codon on the mRNA, while its other end carries an amino acid subunit; in this form the complex is called an amino-acyl tRNA (aa-tRNA).

The three main steps of each cycle of a ribosome are as follows: (i) selection of the cognate aa-tRNA, (ii) formation of the peptide bond between the amino acid brought in by the selected aa-tRNA and the elongating

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protein, and (iii) translocation of the ribosome by one codon. However, some of these steps consist of important sub-steps. Moreover, the branching of paths from a well defined mechano-chemical state can give rise to the possibility of more than one cyclic pathway for a ribosome in a given cycle. Neither of these features was captured by the earlier models [8, 9]. The complete set of states and the pathways for a single ribosome in *our model* are shown in Fig.1. This model may be regarded as an extension of the Fisher-Kolomeisky generic model for molecular motors [10].

The selection of aa-tRNA actually consists of two sub-steps. In the first sub-step, among the tRNAs, which arrive at the rate  $\omega_a$ , non-cognate ones are rejected, at the rate  $\omega_{r1}$ , because of codon-anticodon mismatch. The second sub-step implements a kinetic proofreading mechanism for screening out the near-cognate tRNAs; this sub-step is irreversible and involves hydrolysis of a Guanosine triphosphate (GTP) molecule (at the rate  $\omega_{h1}$ ). Near-cognate tRNAs are rejected at the rate  $\omega_{r2}$ . But, if the tRNA is cognate, the protein gets elongated by the addition of the corresponding amino acid (at the rate  $\omega_p$ ). However, occasionally a near-cognate tRNA escapes potential rejection and the ribosome erroneously incorporates it into the protein (at a rate  $\Omega_p$ ). The subsequent translocation step actually consists of important sub-steps. The first sub-step is a *reversible* relative (Brownian) rotation of the two subunits with respect to each other (with rates  $\omega_{bf}$  and  $\omega_{br}$  along the correct path and with rates  $\Omega_{bf}$  and  $\Omega_{br}$  along the wrong path). In the state labeled by 4 (and 4\*), the tRNAs are in the so-called “hybrid” configuration [7], the details of which are not required for our purpose here. The second sub-step, which is *irreversible*, is driven by the hydrolysis of a GTP molecule; this sub-step leads to the coordinated movement of the tRNAs within the ribosome and the forward stepping of the ribosome by one codon on its mRNA track (at the rates  $\omega_{h2}$  and  $\Omega_{h2}$  along the correct and wrong paths, respectively). Further detailed identification of the states labeled in Fig.1 by the integers [11] is not needed for our purpose here.

Thus, following the selection of a cognate aa-tRNA, the correct pathway is  $1 \rightarrow 2 \rightarrow 3 \rightarrow 4 \rightarrow 5 \rightarrow 1$ . In contrast, if a non-cognate aa-tRNA is picked up, the most probable pathway is  $1 \rightarrow 2 \rightarrow 1$ . However, if the aa-tRNA is near cognate, then the pathway could be either  $1 \rightarrow 2 \rightarrow 3 \rightarrow 1$  (successful kinetic proofreading) or  $1 \rightarrow 2 \rightarrow 3 \rightarrow 4^* \rightarrow 5^* \rightarrow 1$  (incorporation of a wrong amino acid).

In principle, the model does not necessarily require any relation among the rate constants. However, throughout this paper we assume that

$$\omega_{r2} + \Omega_p = C, \quad (1)$$

a constant, because the more stringent the proofreading is, the fewer will be the wrong proteins produced.

We define the fraction

$$\phi = \frac{\omega_p}{\omega_p + \Omega_p} \quad (2)$$

as a measure of translational *fidelity*. The *error ratio*  $\epsilon = \Omega_p/(\omega_p + \Omega_p) = 1 - \phi$ , is the fraction of wrong amino acids incorporated in a protein. We also define the *rejection factor* as

$$\mathcal{R} = \left( \frac{\omega_{r1}}{\omega_{r1} + \omega_{h1}} \right) \left( \frac{\omega_{r2}}{\omega_{r2} + \omega_p + \Omega_p} \right). \quad (3)$$

Note that the average velocity  $V$  of a ribosome is also identical to the average rate of elongation of the protein that it polymerizes. A higher  $V$  results in a larger “quantity” of the protein after a given interval. On the other hand, the parameter  $\phi$  characterizes the “quality” of the final product while  $\mathcal{R}$  characterizes the “effort” of the quality control system in screening out the non-cognate tRNA (including near-cognate tRNA).

### III. RESULTS

Suppose that  $\mathcal{P}_\mu(t)$  is the probability of finding the ribosome in the “chemical” state  $\mu$  at time  $t$ , irrespective of its position on the mRNA track. The obvious normalization condition is  $\sum_{\mu=1}^5 \mathcal{P}_\mu + \mathcal{P}_4^* + \mathcal{P}_5^* = 1$ . Solving the master equations for  $\mathcal{P}_\mu(t)$  in the steady-state, we get the average velocity

$$V = \ell_c(\omega_{h2}\mathcal{P}_5 + \Omega_{h2}\mathcal{P}_5^*) = \ell_c K_{eff} \left( 1 + \frac{\Omega_p}{\omega_p} \right) \quad (4)$$

where  $\ell_c$  is the length of a codon and

$$\begin{aligned} \frac{1}{K_{eff}} = & \frac{1}{\omega_a} \left( 1 + \frac{\omega_{r1}}{\omega_{h1}} \right) \left( 1 + \frac{\omega_{r2}}{\omega_p} \right) + \frac{1}{\omega_{h1}} \left( 1 + \frac{\omega_{r2}}{\omega_p} \right) \\ & + \frac{1}{\omega_p} + \frac{1}{\omega_{bf}} \left( 1 + \frac{\omega_{br}}{\omega_{h2}} \right) + \frac{1}{\omega_{h2}} \\ & + \left( \frac{\Omega_p}{\omega_p} \right) \left[ \frac{1}{\omega_a} \left( 1 + \frac{\omega_{r1}}{\omega_{h1}} \right) + \frac{1}{\omega_{h1}} \right. \\ & \left. + \frac{1}{\Omega_{bf}} \left( 1 + \frac{\Omega_{br}}{\Omega_{h2}} \right) + \frac{1}{\Omega_{h2}} \right] \end{aligned} \quad (5)$$

Note that in the special case  $\Omega_p = 0$ , different terms of  $K_{eff}^{-1}$  are the average time spent by the ribosome in different mechano-chemical states.

The direct transition  $3 \rightarrow 1$  gives rise to “slippage” [12]. Therefore, the coupling between the chemical input and the mechanical output is “loose” [13]. The ratio of the mechanical flux to chemical flux [14] can be taken as a measure of the strength of the mechano-chemical coupling  $\kappa$ :

$$\kappa = \frac{2(P_5\omega_{h2} + P_5^*\Omega_{h2})}{P_2\omega_{h1} + P_5\omega_{h2} + P_5^*\Omega_{h2}} \quad (6)$$

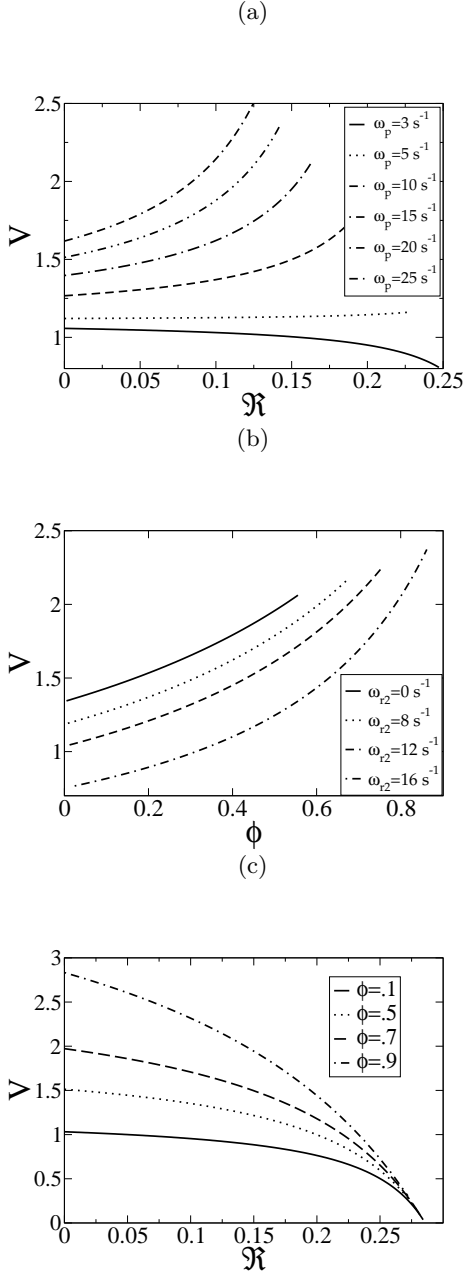


FIG. 2: The average velocity  $V$  of a ribosome (in the units of “codons per second”), i.e., average rate of elongation of a protein (in the units of “amino acids per second”), is plotted against (a) the rejection factor  $\mathcal{R}$ , (for six different fixed values of the parameter  $\omega_p$ ), (b) the fidelity  $\phi$  (for four different fixed values of the parameter  $\omega_{r2}$ ), and (c) the rejection factor  $\mathcal{R}$  (for four different fixed values of the fidelity  $\phi$ ).

so that  $\kappa = 1$  in the limit  $\omega_{r2} = 0$ . Similar quantitative measures of mechano-chemical coupling have been introduced earlier [15] in the general context of motors which can execute “futile” cycles of hydrolysis of nucleotide triphosphates (including GTP). In the steady state of our

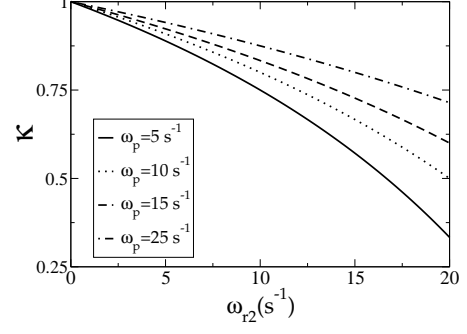


FIG. 3: The mechano-chemical coupling strength  $\kappa$  is plotted against  $\omega_{r2}$  for four different fixed values of the parameter  $\omega_p$ .

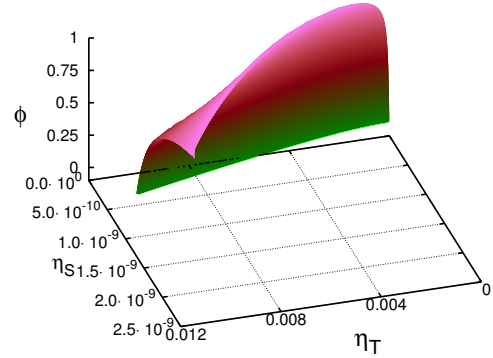


FIG. 4: (Color online) Thermodynamic efficiency  $\eta_T$  and Stokes efficiency  $\eta_S$  are plotted against the fidelity  $\phi$ .

model,

$$\kappa = \frac{2(\omega_p + \Omega_p)}{2(\omega_p + \Omega_p) + \omega_{r2}} = \frac{2(\omega_p + C - \omega_{r2})}{2(\omega_p + C) - \omega_{r2}} \quad (7)$$

The input power consumed in proofreading is

$$P_{in}^p = \left[ \left( \frac{\omega_{r2}}{\omega_{r2} + \omega_p + \Omega_p} \right) \omega_{h1} P_2 \right] \Delta\mu \quad (8)$$

whereas the input power consumed in protein synthesis

$$P_{in}^s = \left[ \left( \frac{\Omega_p + \omega_p}{\omega_{r2} + \Omega_p + \omega_p} \right) \omega_{h1} P_2 + \Omega_{h2} P_5^* + \omega_{h2} P_5 \right] \Delta\mu \quad (9)$$

where  $\Delta\mu$  is the free energy released by the hydrolysis of a single GTP molecule. Thus, the total power input is

$$P_{in} = \left( \frac{\omega_{r2} + 2\omega_p + 2\Omega_p}{\omega_p} \right) K_{eff} \Delta\mu \quad (10)$$

We define the thermodynamic efficiency  $\eta_T$  and the Stokes efficiency  $\eta_S$  by the relations

$$\eta_T = \frac{FV}{P_{in}}, \text{ and } \eta_S = \frac{\gamma V^2}{P_{in}} \quad (11)$$

where  $F$  is the load force and  $\gamma V$  is the phenomenological form of the viscous drag on the ribosome. One of the limitations [4] of definitions (11) is that it implies vanishing thermodynamic efficiency as the external load force vanishes although, in reality, the motor continues to work against the viscous drag.  $\eta_S$  is the appropriate measure of the efficiency of a molecular motor when  $F$  vanishes. From definitions (11)  $\eta_S = (\gamma V/F)\eta_T$ , and using expression (4) for  $V$ , we get

$$\eta_T = \frac{F\ell_c(\Omega_p + \omega_p)}{(\omega_{r2} + 2\omega_p + 2\Omega_p)\Delta\mu} \quad (12)$$

Moreover, we assume the standard form [10] of  $F$ -dependence of  $\omega_{h2}(F)$  and  $\Omega_{h2}(F)$ : the values of these rate constants in the absence of  $F$  are multiplied by the factor  $\exp(-\delta F\ell_c/k_B T)$  where  $0 < \delta < 1$ . For plotting the graphs, we have used the parameter values  $F = 1\text{pN}$ ,  $\Delta\mu = 10k_B T$ ,  $\ell_c = 0.9\text{nm}$ ,  $\delta = 0.5$ ,  $\omega_a = 25.0\text{s}^{-1}$ ,  $\omega_{h1} = 25.0\text{s}^{-1}$ ,  $\omega_{h2}(0) = 25.0\text{s}^{-1}$ ,  $\omega_{br} = \omega_{bf} = 10.0\text{s}^{-1}$ ,  $\omega_p = 25.0\text{s}^{-1}$ , and  $\gamma = 60\text{pN.s.m}^{-1}$ . The parameters  $\omega_{r1}$  and  $\omega_{r2}$  have been varied over a wide range always keeping  $\omega_{r2} + \Omega_p = 20\text{s}^{-1}$ .

The average rate  $V$  of elongation of a protein is plotted against the rejection factor  $\mathcal{R}$  and fidelity  $\phi$  in Figs.2(a) and (b), respectively (note the unit used for plotting  $V$ ). The increase of  $\omega_{r2}$  increases the rejection factor  $\mathcal{R}$ . Therefore, naively, one would expect  $V$  to decrease with increasing  $\mathcal{R}$ . However, because of constraint (1), the increase in  $\omega_{r2}$  is compensated by decreasing  $\Omega_p$ . Therefore, instead of decreasing,  $V$  can increase with  $\mathcal{R}$  provided  $\omega_p$  is sufficiently large (see Fig.2(a)).

Moreover, contrary to naive expectation,  $V$  increases with increasing  $\phi$  as long as  $\omega_{r2}$  is kept fixed (see Fig.2(b)). However, for a given  $\phi$ ,  $V$  decreases with increasing  $\omega_{r2}$ ; this decrease is caused by the increasing frequency of the futile cycles in which a molecule of GTP is hydrolyzed in kinetic proofreading and the aa-tRNA is rejected. In order to emphasize the interplay of  $\mathcal{R}$  and  $\phi$  directly, in Fig.2(c), we plot  $V$  against  $\mathcal{R}$  for several different values of the parameter  $\phi$ . Clearly, for a given  $\phi$ ,  $V$  decreases monotonically with  $\mathcal{R}$ . Similar monotonic decrease in  $V$  with increasing  $\omega_{r2}$  demonstrates that the “slippage” caused by the kinetic proofreading weakens the mechano-chemical coupling  $\kappa$  (see Fig.3).

The variation of the efficiencies  $\eta_T$  and  $\eta_S$  with  $\phi$  is shown in the three-dimensional plot of Fig.4. Taking

cross sections of this diagram parallel to the  $\eta_T - \eta_S$  plane for several different constant values of  $\phi$  (not shown in any figure) we find that  $\eta_T$  increases monotonically with  $\eta_S$ , but the increase is sublinear. This is a consequence of the fact that although  $\phi$  depends only on the ratio  $\Omega_p/\omega_p$ ,  $V$  depends separately also on  $\omega_p$  through its dependence on  $K_{eff}$ .

#### IV. SUMMARY AND CONCLUSION

Intracellular machines which carry out template-dictated polymerization of macromolecules can be regarded as molecular motors [7, 16]. Obviously, as we have shown here, appropriate measures of efficiency can be defined and calculated for such motors. However, the performance of these machines is evaluated better in terms of *fidelity* and rate of polymerization, rather than efficiency and power output. Using our theoretical model for the kinetics of translation, we show that, contrary to the widespread belief, a higher rate of polymerization of a protein does not necessarily compromise the translational fidelity. We have also analyzed the interplay of the “futile cycles” arising from kinetic proofreading and the consequent “looseness” of the mechano-chemical coupling.

For carrying out the calculations analytically, we ignored the possible variations of the rate constants from one codon to another. Therefore, our prediction can be tested *in-vitro* using an artificially synthesized sequence-homogeneous mRNA (i.e., mRNA strand whose codons are all identical, except for the start and stop codons). In the surrounding medium only two species of amino acid subunits should be available, one of these two species is cognate whereas the other is not. The rate constants  $\omega_{r1}$ ,  $\omega_{r2}$ ,  $\Omega_p$ ,  $\Omega_{bf}$ ,  $\Omega_{br}$  and  $\Omega_{h2}$  can be varied by replacing the non-cognate amino acid subunits with another distinct species which is also non-cognate.

The performance of all the intracellular machines of template-dictated polymerization can be characterized and evaluated within the general conceptual framework developed here. However, the results for polymerases [17], which polymerize DNA and RNA, would differ from those of ribosomes because of the differences between their respective mechano-chemical cycles.

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